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A radioimmunoassay for wheat gliadin to assess the suitability of gluten free foods for patients with coeliac disease

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SUMMARY

Coeliac disease is a clinical condition characterised by malabsorption secondary to abnormalities of the small intestine. The condition is known to be exacerbated by wheat gliadin, rye, barley and possibly oats. The only assays that are available for testing for the presence of wheat gluten in foods are double diffusion against rabbit anti-gliadin antiserum and measurement of Kjeldahl nitrogen in products derived from wheat flour. We have developed a radioimmunoassay for wheat gliadin with a detection limit of 1 ng. Nominally gluten free foods based on wheat starch have been shown to contain up to $1{\cdot}9 \times 10^{-2}\%$ wheat gliadin. Bread made from Nutregen wheat starch which has now been withdrawn contains 6·4 mg gliadin per standard 30 g slice. A radioimmunoassay for wheat gliadin could be used to define standards for the suitability of gluten free products based on wheat starch for patients with coeliac disease.

Keywords wheat gluten gliadin coeliac disease radioimmunoassay

INTRODUCTION

The treatment of coeliac disease involves a gluten free diet in which there is avoidance of wheat, rye, barley and in some cases oats. Efforts to characterize the cereal peptide which exacerbates coeliac disease have concentrated on wheat. Each of the major groups of gliadin, the ethanol soluble fraction of wheat flour, has been shown to exacerbate coeliac disease (Jos *et al.*, 1978, 1982; Howdle *et al.*, 1984; Ciclitira *et al.*, 1984). The only assays available for the measurement of wheat gliadin are double diffusion against rabbit anti-gliadin antiserum (Keyser & Mahler, 1973) and measurement of Kjeldahl nitrogen in starch products derived from wheat flour (WHO Codex Alimenterius, 1979). We therefore developed a specific sensitive radioimmunoassay to wheat gliadin.

MATERIALS AND METHODS

Preparation of cereal fractions. Unfractionated gliadin, its alpha, beta, gamma and omega subfractions were derived from Kolibri wheat flour as described by Patey & Evans (1973). Wheat glutenins, albumins and globulins were extracted from a mixture of Timmo and Waggoner wheat flour as previously described (Ciclitira & Lennox, 1983). Rye (Var. Rheidol), barley (Var. Peniarth),

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oat (Var. Porter) and soya (Prewetts Ltd) flours (50 g) were defatted with water saturated *n*-butanol (2 × 100 ml) and ethanol (50% vol./vol.) extracted (10 g/100 ml) to provide rye secalins, barley hordeins, oat avenins and soya prolamins.

The electrophoretic character of the gliadin subfractions by aluminium lactate, starch gel electrophoresis (Woychik, Boundy & Dimiter, 1961) is shown in Fig. 1. The non-gliadin wheat proteins were identified by sodium dodecyl sulphate polyacrylamide slab gel electrophoresis (SDS-PAGE).

Immunization schedule. Unfractionated Kolibri wheat gliadin (100 μg) was dissolved in distilled water and emulsified with 1 ml of Freund's complete adjuvant and injected i.m. into a large White New Zealand rabbit monthly for three consecutive months. Because of a poor antibody response observed by Ouchterlony immunodiffusion, the quantity of immunogen was increased to 300 μg in 1 ml of adjuvant, given monthly for a further 9 months. Serum from the seventh bleed, taken 1 week after the seventh immunization, was used for all the experiments described below.

Preparation of ^{125}I-labelled antigen. The antigen, unfractionated gliadin from the Kolibri strain of wheat which is a heterogenous mixture of proteins with molecular weights ranging from 32 to 58×10^3 daltons was labelled with ^{125}I as previously described (Ciclitira & Lennox, 1983).

The radioimmunoassay. The method for the radioimmunoassay which has been previously reported (Ciclitira & Lennox, 1983) employed the use of the antiserum and iodinated antigen described above.

Preparation of samples for assay of gliadin content. Soluble proteins were dissolved in PBS/BSA at an initial concentration of 100 μg/ml (PBS/BSA: NaCl 8 g/l, KCl 0·2 g/l, Na_2HPO_4 1·15 g/l and KM_2PO_4 0·2 g/l, bovine serum albumin [Armour Pharmaceuticals] 20 g/l, Nonidet P40 [BDH] 5 g/l and sodium azide BDH 1 g/l). To extract proteins from either gluten free foods or flour, these were dried at 50°C, ground to a fine powder and suspended in acetic acid (10 mg in 1 ml of acetic acid, 10 mmol/l). The mixtures were agitated for 2 h at room temparature before centrifugation at 10,000*g* for 5 min; the supernatants were used for subsequent analysis.

RESULTS

Quantification of ct/min. of 25 μl aliquots of unfractionated ^{125}I-gliadin containing approximately 2·5 ng (5×10^3 ct/min) of labelled protein bound to serial dilutions of the antiserum is presented in Fig. 2.

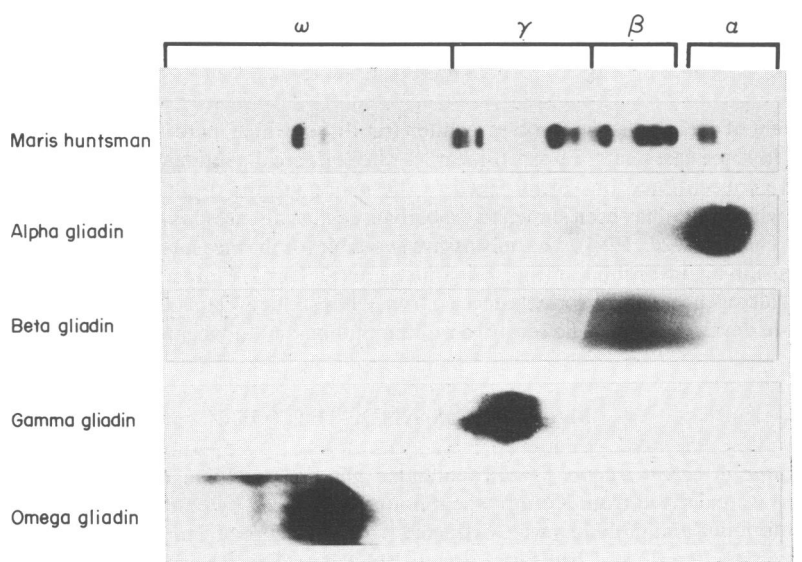

Fig. 1. Electrophoretic patterns of gliadin subfractions used compared to that of a single grain of Maris Huntsman.

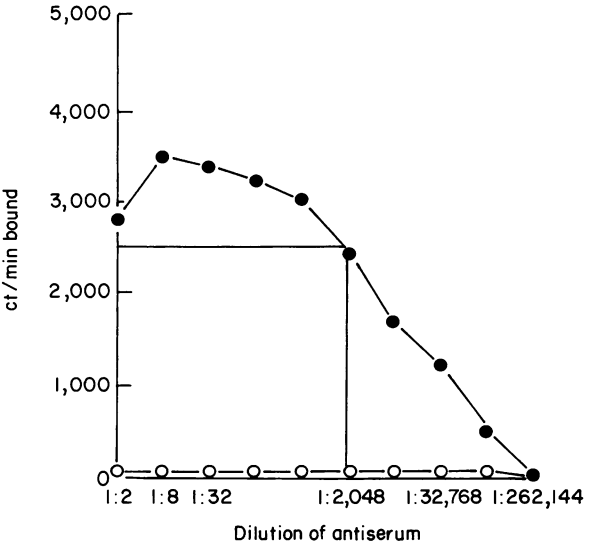

Fig. 2. Radioactivity (ct/min) bound by serial dilutions of the seventh bleed of rabbit antiserum raised against unfractionated gliadin (●) and normal rabbit (○) serum as a control.

Determination of cross-reactivity

Approximately 50% of radioactivity in unfractionated ^{125}I-gliadin was bound when the serum was diluted 1:2,000. The actual percentage varied with the particular radioiodinated sample used. Cross-reactivity was defined as the quantity of material required to produce 50% inhibition of binding between 25 µl unfractionated ^{125}I-gliadin diluted to contain 5×10^3 ct/min and 25 µl of a 1:2,000 dilution of the antiserum.

The quantity of Lowry positive protein that produced 50% inhibition of binding between the radioiodinated antigen and the antiserum was 6 ng unfractionated gliadin, 3 ng beta gliadin, 4 ng alpha gliadin, 8 ng gamma gliadin and > 300 ng omega gliadin (Fig. 3a). There was poor inhibition of binding with wheat glutenins, albumins and globulins (Fig. 3b).

Ethanol extracts of rye (Var. Rheidol), barley (Var. Porter) and oats (Var. Peniarth) gave low levels of inhibition in the competition assay (Fig. 3c). No inhibition of binding was observed with an extract (10 mg in 1 ml of acetic acid 10 mmol) of commercially obtained soya flour. Replacement of radiolabelled gliadin in the competition assay by radiolabelled subfractions resulted in different patterns of cross reactivity: this is to be expected since changing the labelled antigen is likely to change the antibody population bound.

It was decided to use radiolabelled unfractionated gliadin as antigen, because this provided the most sensitive assay for whole gliadin. It seemed unlikely that conventional extraction procedures would differentially remove gliadin components from the flour but the use of labelled whole gliadin would guard against that possibility.

Sensitivity and precision

The sensitivity of the assay was 1 ng unfractionated gliadin. An indication of the precision of the radioimmunoassay was demonstrated by obtaining the same result on testing gliadin subfraction standards on four separate occasions. It is not possible to compare the results with any other assay for estimation of accuracy since none is available.

Testing of nominally gluten free foods for gliadin content

Extracts from commercial wheat flour (an equal mixture of Timmo and Waggoner white wheat flours) and a variety of pre-cooked and cooked gluten free products have been tested. The results are shown in Table 1. The gliadin level measured by our radioimmunoassay in extracts from slices of

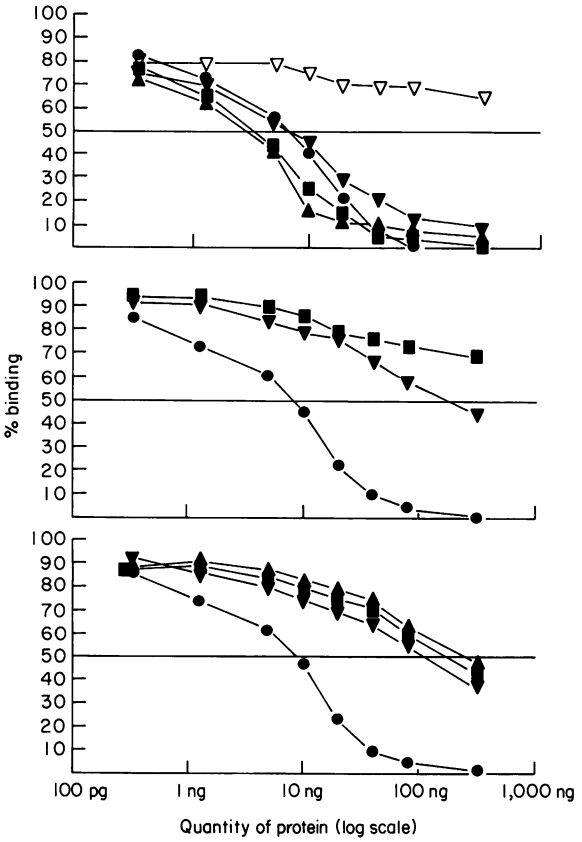

Fig. 3. Radioimmunoassay cross-reactivity curves (antibody dilution 1:2,000) for (a) unfractionated gliadin and its subfractions (● = gliadin, ■ = alpha; ▲ = beta; ▼ = gamma; ▽ = omega) (b) wheat glutenins, albumins and globulins (● = gliadin; ▼ = glutenin, ■ = albumin/globulin) and (c) ethanol extracts of rye, barley and oats (● = wheat, ▼ = rye, ■ = barley, ▲ = oats).

Table 1. Percentage by dry weight of unfractionated gliadin in various nominally gluten free products

Product	% by weight of unfractionated gliadin	Quantity of unfractionated gliadin per standard 30g slice of bread
Nutregen wheat starch	$1{\cdot}9 \times 10^{-2}$	6·4 mg
Juvela gluten free bread mix	$2{\cdot}5 \times 10^{-3}$ $\pm 0{\cdot}255 \times 10^{-3}$ s.d.	Nil detected in commercially available Juvela gluten free bread
Rite diet gluten free flour and bread mix	$1{\cdot}6 \times 10^{-3}$ $\pm 0{\cdot}229 \times 10^{-3}$	0·2 mg
Aglutella pasta	Nil detected	—

both Nutregen and Rite diet gluten free bread were that expected from the respective quantities of wheat starch and bread mix respectively used to make the bread. However, when an extract from a commercially made loaf (Juvela) was tested, no gliadins could be detected by our assay, even though there were detectable amounts of gliadin in three separate batches of bread mix from the same

manufacturer. The presence of wheat gliadin in Nutregen wheat starch has been confirmed by SDS-PAGE when bands which corresponded to wheat gliadin were observed.

DISCUSSION

Alpha, beta, gamma and omega gliadin have been shown to exacerbate coeliac disease by assays involving *in vitro* culture of coeliac jejunal mucosa (Jos *et al.*, 1978, 1982; Howdle *et al.*, 1984) and by clinical *in vivo* challenge studies in coeliac patients (Ciclitira *et al.*, 1984). Early workers found evidence that wheat glutenins, albumins and globulins were not toxic to coeliac patients, although evaluation was by clinical assessment (Van Der Kamer, Weijers & Dicke, 1953) rather than critical measurement. Rye, barley and oats have been reported to exacerbate coeliac disease (Dicke, Weijers & Van Der Kamer, 1953; Rubin *et al.*, 1962; Anand, Piris & Truelove, 1978), although the toxicity of oats remains controversial (Anand *et al.*, 1978). Our observations of low levels of inhibition of binding in the radioimmunoassay described with extracts of rye, barley and oats suggests either low affinity binding to our antiserum of proteins from these cereals, or, that we are not measuring the specific antigenic determinant which exacerbates coeliac disease. Although the shape of the inhibition curve suggests a non-specific effect, no such effect was observed with an extract of soya flour. The poor inhibition of binding of these cereals in our assay means that the absence of cross-reacting substance to our antiserum in a given food product cannot exclude coeliac toxicity.

Antisera were produced to each of the gliadin subfractions but only the antiserum described above was suitable for the measurement of total gliadin. Further assays are required to measure the toxic components within rye, barley and oats. We are currently raising monoclonal antibodies to wheat gliadin that should permit measurement of the specific cereal fractions that exacerbate coeliac disease.

The assay described may be used to determine the sum of gliadin subfractions present in a given food. The poor cross-reaction to omega gliadin could cause inaccuracies in the result obtained, if high levels of this gliadin subfraction were present compared to the other gliadin subfractions. Since in foods the gliadin subfractions are always found together, we feel that the use of this antiserum should not cause inaccuracies in the measurement of total gliadin. The only exception to this may be some gluten free products derived from wheat starch, where it is possible that the ratio of gliadin subfractions differs from that found in wheat flour. However, since omega gliadin is the minor component, comprising normally less than 10% of total gliadin, this is unlikely to affect the amounts of gliadin remaining in products marketed as gluten free.

The amount of gliadin in bread made by us from Nutregen wheat starch accorded with the amount expected from the quantity of Nutregen wheat starch used to make the bread. While we detected gliadin in Juvela bread mix none was detected in commercially baked Juvela bread. Differences in commercial compared to domestic baking may explain these findings.

We have previously shown that 100 mg unfractionated gliadin infused into the duodenum of a patient with coeliac disease produced minimal change in the morphology of serial jejunal biopsies over 8 h (Ciclitira *et al.*, 1984). We have found up to 6·4 mg gliadin per standard 30 g slice of bread made from Nutregen wheat starch, although this product has now been withdrawn. The small amount of gliadin found in other nominally gluten free foods may be important in very sensitive patients with coeliac disease when consumed on a regular basis. This should therefore be considered as a possible cause of poor response of some patients with coeliac disease to a gluten reduced rather than a gluten free diet. The assay described could be used to define standards for the suitability of gluten free foods based on wheat starch to patients with coeliac disease.

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